Colles's fracture should be put up with the wrist moderately flexed. The best position is that in which the hand hangs naturally when it is unsupported and the muscles are relaxed under the anaesthetic. No splint is more efficient or more comfortable than a plaster strip moulded to the flexor surface of the forearm and hand, leaving the fingers free.

The proper use of plaster-of-Paris requires a little practice, but I would submit that the putting up of a Colles's fracture in plaster is within the capacity of any practitioner; it requires less special knowledge and skill than the alteration of an unsuitable splint in order to make it suitable.

An assistant supports the elbow and steadies the limb by making light traction downwards and forwards on the middle fingers. The part is wrapped in a layer of cotton-wool over which an ordinary bandage is applied firmly, but not tightly. A four-inch plaster bandage is placed in warm water, and, when bubbles have ceased to rise, it is lightly squeezed and then applied evenly round and round the limb from the elbow to the middle of the hand. Three or four bandages are applied in like manner, and as soon as the plaster is set it is cut up each side with a sharp scalpel, and the top or dorsal half is removed. The lower half remains as the splint, and it is kept on with an ordinary bandage.

It has been suggested that this fracture should be put up with the forearm supinated, as is usually done with other fractures of the radius and ulna. But the supinated position is much more irksome to the patient, and I doubt whether it is really necessary in this case. Colles's fracture is produced by a fall on the pronated hand and it is impacted in the pronated position. In reducing it by the method described the lower fragment is forced back into its original—that is, normal—position in relation to the upper fragment, and if it is allowed to heal in this position normal movements should follow.

Massage and movements of the fingers are usually begun a day or two after the reduction, and movements of the wrist-joint in about ten days to a fortnight. But these times are of little importance as compared with the complete reduction of the deformity.

In conclusion, is it too much to hope that surgeons in charge of fracture departments allow their house-surgeons and dressers not merely to watch, but actually to do these manipulations with their own hands? Outside the immediate vicinity of large hospitals the great majority of Colles's fractures will be and must be treated by general practitioners, and the actual reduction of a few of these fractures under supervision is worth more to the future practitioner than all the demonstration that can be given.

VALUE OF THE HINTON TEST IN THE SERUM DIAGNOSIS OF SYPHILIS

IN COMPARISON WITH THE KAHN AND WASSERMANN REACTIONS.

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HAVING seen with interest the results of the new Hinton test as applied in the Wassermann Laboratory of the Harvard Medical School and in the pathological laboratory of the Massachusetts General Hospital, Boston, U.S.A., and been fired with Dr. Hinton's enthusiasm during a personal demonstration and discussion of his test, we decided to examine it in a comparative manner with our routine "Wassermann" specimens from the Somerset Hospital, the Peninsula Maternity Hospital, and, in a special series of cases through the kindness and courtesy of Dr. C. Kevin O'Malley, from the venereal disease clinics of the Health Department of Cape Town. One of us (J. H. F.) is responsible for the Hinton tests, the other (E. C. G.) for the Kahn and Wassermann reactions.

THE HINTON TEST.

Our basis for the test is the technique used by Dr. Hinton in June, 1928, which embodies some important modifications (then unpublished) of his original test as appearing in the *Boston Medical and Surgical Journal* of June 16th, 1927 (pp. 993–996).[1] We have used the test with this technique throughout, and have also subjected the procedures to critical experimentation and study, as will appear in the sequel.

The details of the Hinton test, as we have it in a personally annotated reprint with Dr. Hinton's own unpublished modifications (to June, 1928), are briefly set forth as follows:

The test is an "agglutination," by syphilitic serums, of a suspension of cholesterol in *glycerinated* hypertonic saline containing a mere trace of the alcohol-soluble, ether-insoluble extractives of beef muscle. The addition of the glycerol is the main feature of difference from the Kahn, the Sachs-Georgi, Sigma, Meinecke, and other "flocculation" methods of diagnosing syphilitic serums.

Preparation of Reagents.

The "antigen" or "indicator" is prepared from beef muscle (preferred by Hinton to beef heart). The method follows that described by Neymann and Gager,[6] and by Kahn.[2] A small quantity, say 1/2 lb., is freed from fat and connective tissue, chopped and minced, and dried for four or five days on a tray in an oven with a thermostat set at 55° C. It is then powdered, and extracted by vigorous hand-shaking in a stoppered graduate, using ether in the proportion of 1 gram of powder to 4 c.cm. of ether. The process is repeated four or five times until the ether comes away clear, and the residue collected on filter paper and dried. It is then extracted in a glass-stoppered bottle with 95 per cent. alcohol in the proportion of 1 gram of dried residue to 5 c.cm. of alcohol. Extraction is continued for three days at room temperature, and the "indicator" ("antigen") is then filtered off. To every 1 c.cm. of extract is now added 9 c.cm. of a 0.7 per cent. solution of cholesterol in absolute alcohol (prepared several days previously).

The "stock indicator," as Hinton calls it, keeps indefinitely in stoppered bottles of 50 to 100 c.cm. capacity in a dark cupboard at room temperature. The "indicator" actually used for the test is a glycerinated suspension of the cholesterolized antigen *freshly* made up with sodium chloride solution on the day it is to be used. Hinton now makes up a 3 per cent. as well as a 5 per cent. sodium chloride suspension. Two parts of the 5 per cent. (or 3 per cent.) NaCl are added to one part of "stock indicator" pipetted with a clean dry 5 or 10 c.cm. pipette into a 100 c.cm. stoppered graduate. This is shaken vigorously and left for half an hour. Twelve parts of saline are added, the suspension is shaken, and finally, after adding fifteen parts of 50 per cent. glycerol, again shaken thoroughly.

Hinton finds it unnecessary to "inactivate" the serum to be tested, but where routine Wassermann tests are being done it will have been "decomplementized" by heating to 55° C. for half an hour.

The test is carried out in three 10 mm. and 100 mm. (serum) tubes conveniently held in a numbered (Wassermann) rack. This should be of brass and have three shelves to stand immersion without upsetting the serum tubes.

To Tube 1 is added 0.1 c.cm. of serum and 0.5 c.cm. of 5 per cent. NaCl suspension.
To Tube 2 is added 0.3 c.cm. of serum and 0.5 c.cm. of 3 per cent. NaCl suspension.
To Tube 3 is added 0.5 c.cm. of serum and 0.5 c.cm. of 3 per cent. NaCl suspension.

The tubes are shaken thoroughly and placed in a shallow water-bath at 37° C. for six to sixteen hours. The test needs about 1 c.cm. of serum, and the quantities are easy to pipette.

A positive reading consists in a definite "agglutination" of the cholesterol suspension into more or less coarse clumps, with a complete clearing of the medium. A good positive reading in at least one tube (generally the first) constitutes a positive diagnosis of syphilis.

Modifications of Technique.

As a result of our experimentation with the test we submit the following conclusions and modifications of the technique:

1. The saline emulsion of our antigen* when made up with 5 per cent. NaCl is slightly too unstable, tending to "auto-agglutinate," whereas the 3 per cent. NaCl suspension is definitely too stable and too "inert," giving few positives. We found that a 4 per cent. NaCl-glycerol suspension gave uniformly better results, without the needlessly extensive range of sensitiveness intended with the more complicated two-dilutions technique.

* Antigen preparations would appear to vary slightly in sensitivity, as we have noticed in using different batches of antigen.

2. The suspension becomes most sensitive if allowed to stand for half to three-quarters of an hour before use.
3. The 4 per cent. NaCl suspension is added in 0.5 c.cm. amounts to each of the three tubes containing respectively 0.1, 0.3, and 0.5 c.cm. (or 0.1, 0.2, and 0.4 c.cm.) of "inactivated" serum. We use a 20 c.cm. burette graduated in one-twentieths of a cubic centimetre, and find that this makes the addition of 0.5 c.cm. of the suspension to a large series of tubes a rapid and accurate process.
4. To ensure thorough mixing, each tube is shaken with the mouth held loosely between the thumb and forefinger (one tube in each hand); and a rackful of tubes may be shaken by hand in this way with remarkably little trouble and loss of time.
5. Keeping the tubes overnight in the water-bath or incubator at 37° gives the clearest readings. (This would seem to be indispensable, as with a room temperature of 22° to 26° unreliable results were obtained, and preliminary incubation at 55° C. for half an hour to an hour did not improve these results.)

Modified in this way the readings are for the most part clear-cut. Zone phenomena are infrequent and obvious.

Pseudo-Reactions.

A pseudo-reaction is sometimes obtained. For the most part this consists of a fine even flocculation, evident in all three tubes, and tending to be most marked in the tube with the largest amount of serum. On a few occasions this was quite confusing with our use of Hinton's "two-strength" technique, but the fact that there was not much pseudo-agglutination in the 0.1 c.cm. tube caused us to suspect the result in every case. With the 4 per cent. suspension these pseudo-reactions are much less marked and have never given the slightest trouble in reading in 114 cases (vide infra). Hinton emphasizes the clarity of the fluid in which the flocculi float in the case of the true as contrasted with the pseudo-reaction. It may be said that these pseudo-reactions are of slight practical importance and give little or no trouble after a short experience with the tests. Pseudo-reactions, it must be remembered, complicate the Kahn and other techniques, and even the Wassermann reaction is not wholly free from difficulties in reading. As to the validity of the test, Dr. Hinton is, to our certain knowledge, bringing out data from a very large number of observations, and our results support his personally expressed contentions fairly well.

THE KAHN REACTION.

R. L. Kahn, in 1921, appreciating that the "precipitate" in the heterophilic flocculation tests for syphilitic serums was lipoidal in character, as contrasted with the serum globulins in specific immune precipitations, was led to study the variable factors in the reaction, and to evolve a method of stabilizing the conditions of flocculation (precipitation). This method involves:

1. Choice of highly unstable antigen-saline suspension.
2. Optimum concentration of ingredients.
3. Quantitative relation between antigen suspension and serum.
4. Agitation to bring forth the precipitate, especially in weak serums.

The antigen is an alcoholic extract of dried beef heart from which the ether-soluble "non-reactive" extractives have been removed by a careful method of extraction based upon that of Neymann and Gager[6]; 0.6 per cent. of cholesterol is added as experiments indicate that this is the optimum. The optimal amount of saline to add to the cholesterinized antigen is determined by a preliminary antigen titration in which varying quantities of 0.85 per cent. NaCl are added in a standard manner, and note taken of the solubility of the precipitate on the further addition of "test" quantities of saline. The antigen saline proportion we found to be the optimal averaged 1:1, and this is made up ten minutes before the test. We took this titre to indicate correct sensitivity as we had no standard antigen for control-standardizing purposes.

The test proper is conducted with heated serum (56° C. for half an hour being the standard). To 0.05, 0.025, and 0.0125 c.cm. of antigen dilution in agglutination tubes in a suitable (Sigma) rack are added 0.15 c.cm. amounts of heated undiluted serum. We used thorough shaking, by hand, for three minutes to facilitate the reaction. No addition of saline (as recommended by Kahn) was found necessary to make readings clearer. We noted, however, that clear-cut reactions were not immediate, and indeed several hours were necessary before the routine readings could be taken. This led to our leaving the racks at room temperature overnight and reading them along with the Hinton reactions next morning. We consider that this delayed reaction might indicate some lack of sensitivity in our antigen suspension, but conclude that, all other details agreeing with the standard Kahn routine technique, our late (eighteen-hour) readings may be taken as reliable.

Pseudo-reactions were noted in a few cases and were apt to be confusing; our eighteen-hour readings, however, were, on the whole, clear-cut and easy to read. The question of the validity of the results, and the possibility of "false positives," may be referred to a consideration of our tables, and to the experience of other workers.[7] We used cerebro-spinal fluids without the preliminary preparation advocated by Kahn, so as to compare with the Hinton and Wassermann tests. We conclude from our limited observations that this led to very inferior results.

THE WASSERMANN REACTION.

We have employed as a routine the No. 4 method of the Medical Research Committee's Special Report Series No. 14 (1918),[4] which is a standardized method calculated to yield results, both positive and negative, of the highest degree of validity and reliability.[5] Our complement minimum haemolytic dose averaged 1 in 30; and the minimum haemolytic dose of the haemolytic immune body 1 in 1,500.

The serums delivered to us were not always satisfactory, and a number had to be refused on account of haemolysis, gross turbidity, etc. Certain serums with a definite degree of haemolysis were put up and often gave definite reactions. Such of these as were controlled by a subsequent test we have thought fit to include in our series. Anticomplementary serums were ignored, but it is to be noted that in a number of these the Hinton and Kahn tests gave good positives. Wassermann readings were taken immediately after removal of the racks from the water-bath, as it was found that standing even for a short time at room temperature furthered haemolysis and was apt to change weakly positive tubes to negatives (complete haemolysis). Our series of 612 cases includes some 30 odd repeat observations.

TABLE I.—In a comparison of the Hinton and Wassermann tests 612 examinations gave:

Complete agreement in 551 cases (90 per cent.)—	
Both tests negative	384
Both tests positive	167
Doubtful agreement in 15 cases (2.5 per cent.)—	
Hinton negative, Wassermann weak	4
Hinton positive, Wassermann weak	8
Hinton weak, Wassermann negative	3
Disagreement in 46 cases (7.5 per cent.)—	
Hinton positive, Wassermann negative	34
Hinton negative, Wassermann positive	12

TABLE II.—In 460 of these cases, in which the Kahn test was also performed, the Hinton compares with the Wassermann as follows:

Complete agreement in 420 cases (91.3 per cent.)—	
Hinton and Wassermann negative	298
Hinton and Wassermann positive	122
Doubtful agreement in 4 cases (0.9 per cent.)—	
Hinton negative, Wassermann weak	4
Disagreement in 36 cases (7.8 per cent.)—	
Hinton positive, Wassermann negative	31
Hinton negative, Wassermann positive	5

TABLE III.—In a comparison of the Kahn and Wassermann tests in this series of 460 cases we get:

Complete agreement in 428 cases (93 per cent.)—	
Both tests negative	310
Both tests positive	118
Doubtful agreement in 8 cases (1.7 per cent.)—	
Kahn weak, Wassermann negative	4
Kahn negative, Wassermann weak	3
Kahn positive, Wassermann weak	1
Disagreement in 24 cases (5.3 per cent.)—	
Kahn positive, Wassermann negative	15
Kahn negative, Wassermann positive	9

TABLE IV.—Comparing the Hinton and Kahn tests we have:

Complete agreement in 425 cases (92.4 per cent.)—	
Both tests negative	297
Both tests positive	128
Doubtful agreement in 4 cases (0.9 per cent.)—	
Hinton negative, Kahn weak	4
Disagreement in 31 cases (6.7 per cent.)—	
Hinton negative, Kahn positive	6
Hinton positive, Kahn negative	25

TABLE V.—Comparing the three tests for these 460 cases, we have:

Complete agreement in 407 cases (88.5 per cent.)—	
All three tests negative	280
All three tests positive	117
Doubtful agreement in 8 cases (1.7 per cent.)—	
Hinton and Wassermann negative, Kahn weak ...	4
Hinton and Kahn negative, Wassermann weak ...	3
Hinton negative, Kahn and Wassermann weak ...	1
Disagreement in 45 cases (9.8 per cent.)—	
Hinton and Kahn negative, Wassermann positive	4
Hinton negative, Kahn moderately positive, Wassermann positive	1
Hinton and Wassermann negative, Kahn positive	4
Hinton and Kahn positive, Wassermann negative...	11
Hinton positive, Wassermann and Kahn negative...	20
Hinton and Wassermann positive, Kahn negative	5

DISCUSSION.

Comparing these tables, we note that the Hinton and Kahn tests agree fairly closely with the Wassermann reaction, the Kahn slightly more closely than the Hinton. To evaluate the small differences would necessitate clinical data to supplement the serological findings. Satisfactory clinical data were not obtainable in our present series, and we are now at work on a smaller series of selected cases with a view to obtaining a correlation of serological findings with reliable clinical evidence. These results, together with further investigations relating to the fundamental principles underlying the Hinton test, we hope to publish in due course.

We may note in our present series, however, that the Hinton test gives more "positives" than either the Kahn or the Wassermann, and this means either that the test is more sensitive or that it gives a greater number of "false positives." The Kahn also gives a slightly greater number of positives than the Wassermann, and a number of authorities feel that the question of "false positives" with the Kahn calls for some hesitancy in accepting the result in the individual case.[8] It is agreed that false positive Wassermann reactions are very rare under the standardized conditions of the No. 4 method. A ±, and even a + Wassermann, however, demands a repeat, and perhaps a provocative injection of salvarsan, if the clinical evidence of syphilis is insufficient. We have one or two + and − Wassermann reactions, with a negative Hinton, that would need this corroboration of the Wassermann result.

The Hinton test, in our opinion, gives more "false positives" than the Wassermann, but probably (as far as our evidence goes) fewer than the Kahn. This is advanced tentatively, especially in view of the criticisms of our Kahn technique which we have offered above. On the other hand, we feel strongly, on the evidence of a number of the cases having highly suspicious histories, and sometimes giving a positive Kahn reaction also, and occasionally a positive Wassermann on repetition, that the majority of positive Hinton reactions (where the Wassermann is negative) do denote a greater sensitivity of the flocculation test. In the noticeably small number of cases—namely, 5 in our smaller series, and 12 in the larger series—where the Wassermann reaction is positive and the Hinton negative, such evidence as we can command suggests that the inconclusive nature of the ± or + single Wassermann must be considered, as well as the alternative explanation that the Hinton "misses" these cases—that is, gives false negatives. In only two cases, both admitting of doubt, from the incompleteness of the Kahn in one and of the Wassermann in the other, did a positive Kahn accompany a positive Wassermann and the Hinton "miss." Tables I and II, unfortunately, are not comparable on these points, as the Kahn was not performed in quite a proportion of the interesting cases.

With regard to our modification of the Hinton test, we may note that the 4 per cent. NaCl-glycerol indicator was applied to 114 specimens simultaneously with Hinton's "two-strength" indicator, and a greater sensitivity was observed in practically every one of the 36 positives. There were no qualitative differences, save that in two cases with a marked pseudo-reaction in the original Hinton test we obtained a slighter and more easily recognized and discounted reaction with the 4 per cent. suspension.

Our larger series includes 45 cerebro-spinal fluids which give the following comparison:

TABLE VI.

	Hinton Test.	Wassermann Test.
36 cases	negative	negative
1 case	positive	positive
1 ,, ...	weak positive	positive
1 ,, ...	positive	weak positive
1 ,, ...	negative	weak positive
3 cases	negative	positive
2 ,, ...	positive	negative

These few cases rather suggest that the Hinton test is not quite so sensitive with cerebro-spinal fluids, especially as the serums from two of the three cases with positive Wassermann and negative Hinton reactions gave a positive with both tests. The same is true of both of the weakly positive Wassermann reactions. Concerning this point we hope to furnish further evidence at a later date.

The Kahn test, incidentally, was done on 28 of these cerebro-spinal fluids (without the preliminary "concentration" advocated by Kahn), without a single positive result, although including one with both Wassermann and Hinton positive, and two with positive Wassermann and negative Hinton, and one weakly positive Wassermann and negative Hinton, and one with positive Hinton and negative Wassermann. Kahn advocates a preliminary concentration of the precipitating factor in cerebro-spinal fluids by globulin precipitation. Our meagre results would suggest that this is an essential step, and worthy of trial with the Hinton test also.

CONCLUSION.

We have in the Hinton test, especially with the authors' slight modification, a very economical, simple, and highly sensitive test for syphilitic serums. There are indications that the test is somewhat more sensitive and more reliable than the Kahn test. It is a further step towards evolving a simple flocculation technique for routine use. We do not hold that the Hinton test is comparable in reliability with a carefully standardized Wassermann technique, such as the Medical Research Committee's No. 4 method, but it would appear to pick out certain types of cases, especially "treated" cases and the controversial "latent" cases, when the Wassermann test at times fails. In the absence of other evidence, however, one cannot feel that a diagnosis of syphilis is to be made on the Hinton (or for that matter any flocculation reaction) alone, whereas a repeated good positive Wassermann reaction does appear to have that significance.

In short the Hinton, like the Kahn, etc., must be reserved as a supplementary test to the Wassermann. It may be used with the same reserve as applies to other flocculation methods: (*a*) where Wassermann facilities are inadequate or not sufficiently organized (for example, in young countries), or (*b*) in hospitals *as a valuable routine on all cases*, to suggest when a further blood sample should be submitted to the central Wassermann laboratory. Difficulties with pseudo-reactions and false positives still weigh against its use by the general practitioner removed from the convenience of a handy serological laboratory.

We desire to express our appreciation of the interest and advice of Professor William Campbell, through whose courtesy we are enabled to publish these investigations.

REFERENCES.

1 Hinton: *Boston Med. and Surg. Journ.*, June 16th, 1927, p. 993.
2 Kahn, R. L.: *Serum Diagnosis of Syphilis by Precipitation.* Baltimore: Williams and Wilkins Company. 1925.
3 Wassermann, A., Neisser, A., and Bruck, C.: Eine serodiagnostische Reaktion bei Syphilis, *Deut. med. Woch.*, 32, 1906, 745.
4 Medical Research Committee, Special Report Series No. 14, *The Wassermann Test.* No. 1 Report of Special Committee upon the Standardization of Pathological Methods.
5 Ibid., No. 21, *The Diagnostic Value of the Wassermann Test.* No. 4 of same series.
6 Neymann, C. A., and Gager, L. T.: *Journ. Immunol.*, 2, 1917, 571.
7 Osmond, T. E.: (Quoted as an example in the English literature) A Comparison of the Wassermann, Kahn, and Sigma Tests, *British Medical Journal*, March 17th, 1928, p. 440.
8 D'Ath, E. F.: Study of a series of 6,000 comparisons of the Kahn and Wassermann tests on cases with known clinical histories, *British Medical Journal*, 1927, i, p. 625.
9 The American literature on the Kahn reaction has reached considerable dimensions, and is accessible in (1) Kahn's book (loc. cit.); (2) *The Newer Knowledge of Bacteriology and Immunology* by Jordan and Falk (Chicago, 1928); (3) *The Journal of the American Medical Association.*